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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12P 7/18 // C12R 1:72

A1

(11) International Publication Number: WO 88/ 05467

(43) International Publication Date: 28 July 1988 (28.07.88)

(21) International Application Number: PCT/FI87/00162

(22) International Filing Date: 8 December 1987 (08.12.87)

(31) Priority Application Number: 870147

(32) Priority Date: 15 January 1987 (15.01.87)

(33) Priority Country: FI

(71) Applicant *(for all designated States except US)*: FARMOS-YHTYMÄ OY [FI/FI]; PB 425, SF-20101 Turku (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : OJAMO, Heikki [FI/FI]; Kirjurinkuja 3 D 25, SF-02600 Espoo (FI). YLINEN, Lasse [FI/FI]; Isotalontie 16, SF-20310 Turku (FI). LINKO, Matti [FI/FI]; Uudenkirkontie 8, SF-02130 Espoo (FI).

(74) Agent: OY JALO ANT-WUORINEN AB; Laivurinrinne 2 A, SF-00120 Helsinki (FI).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: PROCESS FOR THE PREPARATION OF XYLITOL FROM XYLOSE BY CULTIVATING CANDIDA GUILLIERMONDII

(57) Abstract

Microbiological method for the preparation of xylitol from xylose containing mixtures. The yeast strain Candida guilliermondii VTT-C-71006 is grown, preferably under conditions employing limited aeration, in a xylose rich medium. A very good xylitol yield is obtained by adding to the medium furfural at an amount of about 0.2 to 1.0 g/l. In the fermentation, either a conventional batch process or a so-called fed-batch-process is used.

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Process for the preparation of xylitol from xylose by cultivating Candida guilliermondii.

The object of the present invention is a microbiological process for the preparation of xylitol from xylose containing mixtures using the yeast strain Candida guilliermondii VTT-C-71006.

Xylitol is conventionally prepared on a technical scale by hydrolysing hemicellulose containing substances, for example birch wood, whereby a pentose rich hydrolysate is obtained. The xylose of the hydrolysate can thereafter be hydrogenated catalytically to form xylitol.

Also microbiological methods for the production of xylitol are prior known. In the publication H. Onishi & T. Suzuki, "The Production of Xylitol, L-Arabinitol and Ribitol by Yeasts", Agr. Biol. Chem., Vol. 30, No. 11, p. 1139 to 1144, 1966, is disclosed the microbiological production of xylitol from a xylose containing solution by the yeast species Candida guilliermondii. The initial xylose concentration of the solution used as raw material was 10 % by weight. The yield of xylitol was 40.3 %.

The strain Candida guilliermondii VTT-C-71006 is deposited in the strain collection at National Collection of Yeast Cultures (NCYC), Norwich, England, on 17th December 1986 under no NCYC-1644.

Candida guilliermondii VTT-C-71006 exhibits the following morphological and physiological properties.

Morphological characteristics:

Macroscopic morphology:

Soft, shiny, white/light yellow colonies with a diameter of 1 to 2 mm on malt extract (3 g/l)-yeast extract (3 g/l)-glucose (10 g/l)-peptone (5 g/l)-agar (20 g/l) (MYGP-agar).

Microscopic morphology:

The cells immobile in MYGP-solution, somewhat oval, dimensions 2-5 x 7-3 /um. Under some conditions formation of pseudomycelium.

Physiological characteristics:

The physiological properties were determined as described in the book N.J.W. Kreger-van Rij, "The yeasts - a taxonomic study", 3rd edition, Elsevier Science Publishers B.V. Amsterdam 1984, p. 76-.

Fermentation:

glucose	+
sucrose	+
raffinose	?
galactose	?
maltose	-
lactose	-
xylose	-

Assimilation:

glucose	+
sucrose	+
maltose	+
cellobiose	+
raffinose	+
xylose	+
lactose	-
starch	-
nitrate	-
inositol	-

According to the process of the invention, cultivation is carried out by cultivating the strain Candida guilliermon-

dii VTT-C-71006 preferably under aerated conditions in a xylose containing medium having a xylose concentration varying from 5 to 50 % by weight at a temperature of 20 to 40°C for a period ranging from 20 hours to 10 days. After the removal of the yeast the xylitol formed during the cultivation may be recovered by any known manner.

Suitable raw materials are any xylose containing mixtures, for example the xylose rich mixtures obtained from the hydrolysis of hemicellulose containing materials.

In the fermentation solution as the N-source for the yeast different ammonium salts may be used and as a growth factor source for example yeast extract, yeast produced in the process or extract of plant origin. Essential is the restriction of the oxygen supply to the yeast. By adjusting the stirring and aerating conditions to an optimum, xylitol is obtained at an amount as high as 78 %. The process is slowed down at very high initial xylose concentrations. The process may, however, be accelerated compared to a normal batch process even by 40 % by using so called fed-batch cultivation, which means that during the process, nutrients are added to the fermenter. In our tests the cultivation was carried out using different xylose concentrations and different aeration efficiences in both a fermenter and in agitated cultures.

The composition of the medium was

xylose	40 to 300 g/l
yeast extract	10 g/l
Bacto peptone	3 g/l.

In our tests it was surprisingly discovered that the addition of furfural favourably affected the yield of xylitol. Previously the presence of furfural in microbiological processes has been considered purely disadvantageous as

furfural inhibits the growth of yeast. The inhibition of the growth of the yeast is as such desirable in processes of this kind, as the growth of yeast consumes raw materials which is manifested in a decrease of the amount of xylitol. Surprising was, however, the fact that furfural did not completely inhibit the action of the yeast, but acted as a factor promoting the production of xylitol. According to literature, furfural usually disturbs both the growth and the fermentation process (for example N. Banerjee et al., European J. Appl. Microbiol. Biotechnol. 1981, 11:226-228)).

According to this process it was discovered that the growth of yeast and the subsequent reduction of the xylitol yield may be limited by adding furfural to the fermentation solution at an amount of 0.2 - 1.0 g/l.

The use of furfural for the promotion of microbiological processes is not limited to the production of xylitol from xylose by means of the process described above. It is evident that this observation may be applied also to other microbiological processes, the object of which is not the production of the microbe as such but the production of substances independent of the microbial growth, for example in biotransformations.

During the cultivation, the yeast concentration was determined by measuring the turbidity, the xylose concentration by determining the concentration of reducing substances and the formation of xylitol was determined qualitatively by using thin layer chromatography and quantitatively by using liquid chromatography or an enzymatic method.

The process according to the invention exhibits many advantages with respect to the known processes. Of these may be mentioned for example:

- High xylitol yield, 50 to 78 %, depending on the xylose concentration. The process mentioned earlier and belonging to the state of art gave a xylitol yield of only 40.3 % although the initial xylose concentration was only 10 %. When using our method and starting from the corresponding initial xylose concentration, the yield obtained was 74 %.

- It is possible to use high initial xylose concentrations. This factor is of great technical and economical importance. With an initial xylose concentration of the raw material of 300 g/l a xylitol yield as high as 50 % was obtained.

- The xylitol produced with this method is pure and is thus quite suitable for use in foodstuffs. Xylitol produced by means of catalytic hydrogenation may contain residues of toxic catalyst. The method according to the invention is also more economical, as the catalysts are expensive and are consumed in the process.

- This method is also very specific, as the yeast strain Candida guilliermondii VTT-C-71006 converts xylose only to xylitol. In the catalytic hydrogenation, besides xylitol by-products may be obtained, which makes purification more difficult.

In the following examples the process according to the invention is described in more detail.

Example 1

The strain Candida guilliermondii VTT-C-71006 is grown aseptically while agitating in a solution volume of 100 ml in a 250 ml vessel. The growth temperature is 28°C. The medium contains 80 g/l xylose, 10 g/l yeast extract and 3 g/l Bacto peptone.

The pH of the medium was adjusted to a value of 5.0. As the inoculum 1 ml of a culture grown overnight on MYGP-medium is used. After two days of cultivation, the xylose has been consumed and the xylitol concentration is 50 g/l. The xylose was determined as reducing sugars and the xylitol using liquid chromatography. The xylitol yield was thus 62.5 %.

Example 2

The method according to the Example 1 was repeated, the initial xylose concentration being 300 g/l. After 6 days the xylose was consumed completely and the xylitol concentration was 150 g/l, which corresponds to a yield of 50 %.

Example 3

The method described in the Example 1 was repeated, but furfural was added to the medium at an amount of 0.6 g/l. The xylitol concentration after 53 hours cultivation was 61 g/l (yield 76 %).

Example 4

The strain mentioned in the Example 1 is grown aseptically in a fermenter in a medium containing 100 g/l xylose, 10 g/l yeast extract and 3 g/l Bacto peptone. The pH of the medium was adjusted to a value of 5.0 and the stirring speed to 400 rpm and the aeration to about 0.5 l/min. After 50 hours of cultivation the xylose was totally consumed and the xylitol concentration was 74 g/l (yield 74 %).

Example 5

The process of Example 4 was repeated but using 250 g/l as the initial xylose concentration. After cultivating for 160 hours, the xylose was completely consumed and the xylitol concentration was 138 g/l (yield 55 %).

Example 6

The strain mentioned in the previous example is grown in a fermenter as a fed-batch-culture. The initial volume of the culture solution was 60 % of the end volume and its composition was 100 g/l xylose, 10 g/l yeast extract and 3 g/l Bacto peptone. Cultivation was started as in the Example 4, but when the xylose concentration had decreased to a value of 40 g/l, the introduction of a xylose solution at a concentration of 350 g/l was begun. By means of the addition the xylose concentration in the fermenter was kept at 40 g/l. When the final volume was reached the addition was stopped. After cultivating for 120 hours the xylose was completely consumed and the xylitol concentration was 164 g/l, which corresponds to a xylitol yield of 78 %.

Claims

1. Process for the preparation of xylitol from xylose containing mixtures, c h a r a c t e r i z e d in that the yeast strain Candida guilliermondii VTT-C-71006 is cultivated, advantageously under conditions of limited aeration, in a xylose containing medium at a temperature of 20 to 40°C for a period which varies between 20 hours and 10 days, whereafter the xylitol formed in the medium is recovered.

2. Process according to the claim 1, c h a r a c t e r i z e d in that in order to restrict the growth of the yeast and thus to improve the xylitol yield, furfural at an amount of 0.2 to 1.0 g/l is added to medium.

3. Process according to the claim 1 or 2, c h a r a c t e r i z e d in that as the medium is used a xylose containing, pentose rich mixture of sugars obtained from the hydrolysis of hemicellulose containing materials.

4. Process according to any of the previous claims, c h a r a c t e r i z e d in that in the fermentation a fed-batch process is used.

INTERNATIONAL SEARCH REPORT

PCT/FI87/00162

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC [4]

C 12 P 7/18, // C 12 R 1:72

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 P 7/02,/18; C 12 R 1:72; C 12 C 11/00
US Cl	195:37,43; 435:158,255

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE, NO, DK, FI classes as above

Data base search: WPI, WPIL, CA, BIOSIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Chemical Abstracts, Vol. 94 (1981), abstract No 94:207106a, Biotechnol. Lett. 1981, 3(3), 125-30 (Eng).	1-4
Y	Chemical Abstracts, Vol. 102 (1985), abstract No 102:3048g, Mikrobiologiya. 1984, 53(5), 803-8 (Russ).	1-4
Y	US, A, 3 619 369 (NODA INSTITUTE FOR SCIENTIFIC RESEARCH) 9 November 1971 & CA, 1939035	1-4
X	Agr. Biol. Chem. Vol. 30, 1966, Hiroshi Önishi, "The Production of Xylitol. L-Arabinitol and Ribitol by Yeasts", p 1139-1144	1-4
Y	Chemical Abstracts, Vol. 72 (1970), abstract No 41685g, Appl. Microbiol. 1969, 18(6), 1031-5 (Eng).	1-4

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1988-03-08	1988 -03- 09
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